APSSPT 3 - Röper - "Hoffmann" analytes and cigarette smoke in vitro toxicity revisited - How do the data compare?

Authors: RÖPER W.; WIECZOREK R.; INTORP M.

"Hoffmann" analytes and cigarette smoke in vitro toxicity revisited - How do the data compare?

Röper W., Wieczorek R., Intorp M.
Reemtsma Cigarettenfabriken GmbH, Imperial Tobacco Group, Scientific & Technical Affairs, Albert-Einstein-Ring 7, 22761 Hamburg, Germany

Summary

Mainstream smoke *in vitro* cytotoxicity and genotoxicity of a series of experimental cigarettes made up from single grade Virginia and Burley tobaccos and an Oriental Blend were determined. Smoke condensates, whole smoke or vapour phase extracts were tested, employing standard techniques such as the Neutral Red Uptake viability assay, the MTS cell proliferation assay (both with a human liver carcinoma cell line) and the AMES microbial reverse mutation assay (with *Salmonella typhimurium* TA98). In parallel, a set of chemical analytes with potential toxicological relevance, the so-called Hoffmann Analytes, was determined in mainstream smoke as well. In order to evaluate the contribution of single analytes or groups of analytes to overall smoke toxicity, the level of some selected "Hoffmann's" in mainstream smoke of different experimental cigarettes was compared with the smoke dose yielding 50% toxicity in smoke treated cells (EC 50) and the dose of condensate showing mutagenic effects in bacteria, respectively. Additionally, Neutral Red cytotoxicity data of single "neat" smoke constituents were compared with their levels in mainstream smoke and the corresponding smoke toxicity data. There were significant differences of toxicity amongst tobacco grades of the same and different tobacco varieties. Generally, on a per mg 'tar' basis, Burley cigarettes had the highest mutagenic potential, followed by Virginia (40% less) and Oriental (60% less) cigarettes; condensate cytotoxicity was slightly lower in Burley, compared to Virginia and Oriental cigarettes. Whole smoke and vapour phase extracts of Burley exhibited significantly less cytotoxicity than did those of Virginia and Oriental cigarettes. As regards smoke toxicity and levels of "Hoffmann's", some statistical associations were found with vapour phase compounds rather than particulate matter constituents, indicating that mechanisms of cigarette smoke genotoxicity and cytotoxicity are too complex to be attributed to the effects of some selected smoke constituents.

Introduction and Rationale

Some two years ago, in their outstanding presentation given to the 2002 CORESTA congress (3), Charles R. Green and Alan Rodgman critically examined the listing and prioritisation of toxic chemicals in cigarette mainstream smoke. They pointed to a number of limitations in existing knowledge which call into question the reliability of such listing strategies. Today,

our industry is facing existing and upcoming regulations in terms of testing of emissions other than tar, nicotine and CO, and biological testing. Indeed, some regulators require the measurement of 44 smoke analytes with potential toxicological relevance, the Hoffmann Analytes (Table 1).

Biological testing for example can be carried out by employing *in vitro* methods as recently outlined by a CORESTA task force (5). Some of these *in vitro* methods have already been used by the tobacco industry for many years, and papers have been published on smoke (2) and single substance (1) toxicity, some papers also taking into account changes in smoke chemistry and toxicity caused by smoking regimes other than ISO (4).

Table 1: List of Hoffmann Analytes, compounds in bold letters were analysed for this study

NFDPM ('Tar')	**Isoprene**	Propanal	
Nicotine	Acrylonitrile	Butanal	
CO	Quinoline	**Crotonaldehyde**	
Benzo(a)Pyrene (BaP)	Pyridine	**NNK**	
Nitric oxide	Phenol	**NNN**	
Hydrogen Cyanide (HCN)	**m/p-Cresol**	**NAT**	
Ammonia	**o-Cresol**	**NAB**	
Benzene	**Formaldehyde**	**4-Aminobiphenyl (4-AB)**	
Toluene	**Acetaldehyde**	**3-Aminobiphenyl (3-AB)**	
Styrene	**Acetone**	**2-Naphthylamine (2-NA)**	
1,3-butadiene	**2-Butanone (MEK)**	**1-Naphthylamine (1-NA)**	

Although some of the Hoffmann's are listed by IARC as carcinogens, probable or possible human carcinogens (cat 1, 2A, 2B), and some are known to be cytotoxic, we must bear in mind that their levels in tobacco smoke may be as low as ng/cigarette. Thus, although biologically active at elevated concentrations in a single biological test, their levels in smoke may be too low to significantly influence the outcome of any test conducted with tobacco smoke. The objective of this study was to compare chemical analytical data with biological test data from experimental cigarettes expected to significantly differ in smoke emissions in order to assess whether or not elevated levels of smoke constituents might increase overall toxicity of the smoke.

Experimental Cigarettes

Ten experimental filter tipped cigarettes manufactured from single grade Virginia and Burley tobaccos and an Oriental Blend cigarette according to Table 2 were used for this study.

Table 2: Experimental cigarettes (King Size, filtered, non-ventilated)

Code	Type	Country of origin	single grade	'Tar' *mg/cig*	CO *mg/cig*	Nicotine *mg/cig*

V-1	Virginia	Zimbabwe	x	15.6	16.3	1.95
V-2	Virginia	Brasil	x	10.2	12.8	1.04
V-3	Virginia	Brasil	x	12.8	14.1	1.38
V-4	Virginia	Brasil	x	17.3	16.2	2.51
V-5	Virginia	USA	x	19.3	16.6	2.07
B-1	Burley	Italy	x	11.3	15.8	0.73
B-2	Burley	Malawi	x	10.3	15.4	0.53
B-3	Burley	Guatemala	x	11.0	15.7	0.82
B-4	Burley	Guatemala	x	13.4	16.8	2.20
B-5	Burley	USA	x	11.7	17.3	1.56
OR	Oriental		blend	17.6	15.1	0.80

Biological Testing

In vitro testing was carried out following in part the recommendations of the "*In vitro* toxicology" Task Force of CORESTA (5), including the AMES assay (TA 98 only) and the Neutral Red Uptake assay, but doing without the micronucleus assay.

Biological Test Battery

- AMES microbial mutagenicity assay with strain TA 98 (*Salmonella typhimurium*) and S9 metabolic activation (rat, aroclor), plate incorporation assay (7).
- Neutral Red uptake (NRU, 6) toxicity test with HEP-G2 human (liver hepatoma, ATCC HB-8065) cell line, cultured in serum-free medium (cell exposure in 96-well MTPs).
- MTS cell proliferation test with HEP-G2, using MTS tetrazolium compound (Owen's reagent, 8,9).

Preparation of 'Smoke' for Testing

Cells were exposed to smoke condensate and aequous extracts of vapour phase or whole smoke.

- Condensates were collected on 92 mm Cambridge filter pads by smoking 20 cigarettes according to the ISO standard. Filters were then extracted with 20 ml DMSO. Aliquots of this stock solution were diluted, as appropriate, and used for biological testing (AMES, NRU, MTS).
- Vapour phase or whole smoke extracts were prepared using a single port smoking machine (35 ml puff, 2 seconds, puff interval 58 seconds). Whole smoke or vapour phase of three cigarettes was consecutively bubbled through an impinger containing 15 ml of serum-free culture medium. Cells were exposed to various aliquots of these extracts (NRU, MTS).

Testing of 'neat' smoke constituents

- Commercially available samples of acetaldehyde, formaldehyde, and acrolein were used to determine single substance cytotoxicity (aequous solutions).

Data Evaluation

- AMES plate count data were expressed as revertants per mg condensate and revertants per cigarette, respectively, and normalized against the mean value of all samples.
- Data from Neutral Red Uptake (NRU) and MTS assays were plotted as dose-response curves, cytotoxicity vs. dose (µg tar/mL, 'cigarette equivalents' per litre or puffs per litre), compared to untreated control cells. From these curves, the smoke dose for 50% cytotoxicity (EC 50) was calculated. EC50 is inversely related to cytotoxicity; thus, 1/EC 50 was calculated as well and normalized using the mean of all samples as a reference point.

Analytical Testing

Selected mainstream smoke Hoffmann-Analytes of the experimental cigarettes (Table 1) were determined in one laboratory (Imperial, Hamburg) using internally validated in-house methods. Data were expressed as 'analyte per cigarette' or 'analyte per puff'. For data comparison, all results were normalized, i.e. expressed in relative terms (data mean = 1.00).

Comparison of Biological Data with 'Hoffmann' Data

Normalized biological and analytical data were compared by regression analysis on a per puff or a per cigarette basis.

Results and Conclusions

Normalized condensate toxicity data are given in Table 3, data for vapour phase and whole smoke extracts are listed in Table 4.

Table 3: Relative (normalized) mutagenicity (TA 98) and cytotoxicity (NRU) data of cigarette smoke condensates

Code	mutagenicity per mg 'tar	mutagenicity per cigarette	cytotoxicity per mg 'tar' *1/EC 50*	cytotoxicity per c *1/EC 50*
V-1	0.79	0.94	1.11	1.37
V-2	0.67	0.55	1.00	0.84
V-3	0.62	0.61	1.03	1.05
V-4	0.96	1.32	1.00	1.43
V-5	0.96	1.41	1.07	1.62
B-1	1.47	1.18	1.00	0.82
B-2	1.19	0.87	0.93	0.70
B-3	1.26	0.95	1.03	0.80
B-4	1.32	1.38	0.89	0.96
B-5	1.24	1.10	0.94	0.86
OR	0.52	0.69	1.05	1.41

Table 4: Relative (normalized) cigarette specific cytotoxicity data of vapour phase (MTS

assay) and whole smoke (NRU assay) extracts

	vapour phase cytotoxicity		whole smoke cytotoxicity	
	1/EC 50		*1/EC 50*	
Code	NRU	MTS	NRU	MTS
V-1	1.71	2.00	1.43	1.93
V-2	1.63	1.89	1.36	1.62
V-3	1.93	2.40	1.88	2.03
V-4	1.54	1.89	2.14	2.03
V-5	1.48	1.89	2.00	1.93
B-1	0.58	0.52	0.71	0.66
B-2	0.74	0.76	0.75	0.71
B-3	0.74	0.70	0.63	0.63
B-4	0.65	0.62	0.79	0.70
B-5	0.72	0.59	0.57	0.57
OR	1.66	1.84	1.43	1.45

Condensate toxicity (Table 3)

AMES mutagenicity on a per mg tar basis was lowest in Oriental, intermediate in Virginia and highest in Burley tobaccos, on a per cigarette basis (influenced by tar levels as well) considerable variation was observed between different grades of the same type, some Virginia grades exhibiting more overall mutagenicity than Burley grades. Per mg tar NRU cytotoxicity was similar for all condensates, only slightly lower for the Burley grades. Cigarette specific cytotoxicity on average was lower for Burley grades than for Virginia and Oriental grades.

Smoke extract cytotoxicity (Table 4)

Whole smoke extracts were considerably more toxic than vapour phase extracts (vapour phase EC50s by a factor of three to four higher than whole smoke EC 50s, data not shown), indicating a substantial trapping of particulates when preparing the extracts. Marked differences between grades were observed as regards cigarette specific vapour phase and whole smoke NRU and MTS data. Burley grades were significantly less toxic than Virginia and Oriental grades (EC 50s of Burley grades at least twice those of Virginia/Oriental grades).

Levels of single smoke constituents and cytotoxicity

In order to assess the impact of selected vapour phase Hoffmann analytes on vapour phase extract cytotoxicity their theoretical concentration in the extracts (at the final dose the cells were exposed to at EC 50, assuming 100% trapping in the impinger) were calculated and compared with the EC 50 for the neat substance, and for three analytes these data are given in table 5.

It is interesting to note that from these data smoke acrolein alone might readily explain the observed cytotoxicity, whilst the levels of acetaldehyde and formaldehyde are orders of magnitude below those needed to exhibit any cytotoxicity.

Table 5: EC 50 of three selected smoke constituents in the Neutral Red and MTS Assay, compared to their theoretical concentration in vapour phase extracts @ EC 50 of vapour phase

Analyte	**Acetaldehyde** mg/L		**Acrolein** mg/L		**Formaldehyde** mg/L	
Sample	NRU	MTS	NRU	MTS	NRU	MTS
V-1	9.78	5.71	1.48	0.86	1.04	0.61
V-2	7.65	4.56	1.15	0.68	0.90	0.54
V-3	9.07	4.87	1.35	0.73	1.20	0.65
V-4	10.95	6.06	1.50	0.83	0.78	0.43
V-5	10.74	5.71	1.66	0.88	0.95	0.50
B-1	20.66	15.42	1.96	1.46	0.31	0.23
B-2	20.37	13.26	1.76	1.16	0.35	0.23
B-3	18.12	12.24	1.89	1.27	0.33	0.22
B-4	18.55	13.06	1.99	1.40	0.28	0.20
B-5	15.39	13.05	1.69	1.43	0.27	0.23
OR	8.55	5.24	1.43	0.88	1.32	0.81
neat substance EC 50	561	630	2.55	0.94	44.8	19.4

Levels of analytes and genotoxicity/cytotoxicity

A complete listing of Hoffmann Analyte levels for the eleven experimental cigarettes can be found in Appendices 1 and 2 (normalized data including the mean of all). The means of all normalized data show much less variance than most data for a given single analyte, indicating that lower levels of some analytes are linked with higher levels of others. The highest 'overall level' of Hoffmann Analytes is observed in sample B-4, the lowest in B-2. B-4 is ranking close to top as regards mutagenicity, but better than average in all other tests. B-2 is better than average in all biological tests, but similar to B-3 (with a higher Hoffmann Analyte level). Anything else?

Appendix 3, on a per puff basis, summarises the correlation coefficients calculated from the analytical and toxicological data (linear regression). It is beyond the scope of this paper (and beyond the expertise of its authors) to analyse whether significant correlations may indicate any causal or mechanistic effects. Some of those obviously do not make sense and may be 'confounded' by something else. It is, however, interesting to note that a higher level of some analytes in a puff does not necessarily result in a higher toxicity of this puff, as measured with our test battery. Indeed, according to statistics, increasing levels of hydrogen cyanide will decrease puff specific whole smoke cytotoxicity, as will increasing levels of nitric oxide for whole smoke and m/p-cresol for vapour phase. Higher levels of formaldehyde, catechol and acrolein indicate increased toxicity but less AMES mutagenicity; levels of nitrogen containing compounds (TSNA, aromatic amines, nitric oxide) positively correlate with mutagenicity, indicating the well known importance of tobacco nitrogen for TA 98 mutagenicity. BaP in fact is inversely correlated with the AMES data (its dose in condensate being too low for response in TA 98), but positively correlated with NRU and MTS, Virginia

being less mutagenic but more cytotoxic than Burley.

In conclusion, the contribution of specific Hoffmann Analytes to tobacco smoke *in vitro* toxicity is (perhaps with the exception of acrolein) by no means clear (and will be an issue for future research), indicating that mechanisms of cigarette smoke genotoxicity and cytotoxicity are too complex to be attributed to the effects of some selected smoke constituents.

Anyway, regulators will have to decide on appropriate testing procedures for tobacco products, whether chemical analytical or biological tests, in order to further evaluate toxicological properties of tobacco products.

References

1. Bombick, DW, Doolittle DJ (1995): The role of chemical structure and cell type in the cytotoxicity of low-molecular-weight aldehydes and pyridines. **In Vitro Toxicol. 8,** 349-356.
2. Bombick DW (2002): Assessment of tobacco smoke by in vitro cell toxicity assays. **Recent Advances in Tobacco Science 28**, 104-136.
3. Rodgman A, Green CR (2003): Toxic chemicals in cigarette mainstream smoke - Hazard and hoopla. **Beitr Tabakforsch Int 20**, 481-541.
4. Roemer E, Stabbert R, Rustemeier K et al. (2004): Chemical composition, cytotoxicity and mutagenicity of smoke from US commercial and reference cigarettes smoked under two sets of machine smoking conditions. **Toxicology 195**, 31-52.
5. CORESTA In Vitro Toxicology Task Force: The rationale and strategy for conducting in vitro toxicology testing of tobacco smoke. Final Report 2004.
6. Borenfreund E., Puerner J. A. (1985): Toxicity determined in vitro by morphological alterations and neutral red absorption. **Toxicol Lett 24,** 119-124.
7. Maron DM, Ames BN (1983): Revised methods for the *Salmonella* mutagenicity test. **Mutat Res 113**, 173-215
8. Promega Technical Bulletin No. 169 (http://www.shpromega.com.cn/tb169.pdf)
9. Riss TL, Moravec, RA (1992): Comparison of MTT, XTT, and a novel tetrazolium compound MTS for in vitro proliferation and chemosensitivity assays. **Mol Biol Cell 3**, Suppl 184a

Acknowledgements

The technical assistance of Elke Burmester, Birgit Johnston, Edmond Kahl and Catrin Lietz is gratefully acknowledged. Chemical analyses were carried out at Imperial Tobacco's Special Analytes Laboratory. Thanks to Christian Müller and his team for the data.

Appendix

Appendix 1:

Yields of selected Hoffmann Analytes in mainstream smoke of experimental cigarettes

Analyte	V-1	V-2	V-3	V-4	V-5	B-1	B-2	B-3	
BaP ng/cig	12.8	15.6	16.8	20.5	15.8	5.2	3.7	5.2	
Nitric oxide µL/cig	45	78	43	66	54	251	86	301	
HCN µg/cig	139.7	121.8	137.0	144.9	235.7	120.1	88.1	156.6	1

Benzene µg/cig	68.7	62.8	67.9	70.8	66.9	51.7	44.0	43.7	5
Toluene µg/cig	104.7	91.1	105.2	116.4	118.1	100.8	77.7	82.3	9
Isoprene µg/cig	575.3	398.4	484.5	792.9	879.2	273.5	343.6	292.6	4
Phenol µg/cig	26.1	14.1	19.7	26.8	31.9	13.5	11.6	11.1	2
m/p-cresol µg/cig	12.3	8.5	10.0	14.5	15.8	9.1	7.6	8.9	1
o-cresol µg/cig	4.4	3.1	3.6	4.7	5.3	2.8	2.6	2.8	
Formaldehyde µg/cig	73.9	75.2	101.6	53.4	64.3	8.3	12.2	11.5	
Acetaldehyde µg/cig	694.3	640.1	766.9	749.5	730.3	564.9	707.3	640.6	5
Acetone µg/cig	321.9	304.4	299.3	271.5	294.2	298.4	275.7	325.2	3
2-Butanone µg/cig	67.4	64.9	62.1	58.1	66.0	57.6	53.0	54.9	5
Crotonaldehyde µg/cig	26.4	23.5	26.2	25.2	30.8	17.7	19.5	19.0	1
NNK ng/cig	31.5	28.1	15.7	29.8	155.4	88.6	133.9	159.3	1
NNN ng/cig	30.2	25.3	7.1	34.0	78.2	236.9	87.5	661.4	1
NAT ng/cig	39.5	36.0	13.6	63.5	112.0	182.8	51.0	503.3	9
NAB ng/cig	5.6	5.0	5.0	10.0	15.2	20.6	8.8	59.9	1
4-AB ng/Cig	1.6	1.5	1.1	2.1	2.4	3.7	2.0	1.8	
3-AB ng/cig	1.9	1.9	1.3	2.6	3.2	3.5	2.3	2.3	
2-NA ng/cig	11.8	10.3	7.4	16.1	19.1	12.0	9.9	7.5	1
1-NA ng/cig	15.1	15.9	11.3	22.9	24.9	15.5	15.1	9.1	1
Resorcinol µg/cig	2.1	0.8	1.7	2.7	2.0	0.6	0.8	0.4	
Hydroquinone µg/cig	84.0	31.9	77.0	74.2	76.3	36.7	71.2	19.7	4
Catechol µg/cig	98.2	50.4	85.5	99.2	97.0	27.6	34.3	19.1	3
Acrolein µg/cig	105.0	95.8	114.5	102.4	112.8	53.6	61.6	66.7	6

Appendix 2:

Normalized per cigarette yields of selected Hoffmann Analytes in mainstream smoke, including average of all Hoffmann's

Analyte	V-1	V-2	V-3	V-4	V-5	B-1	B-2	B-3	
BaP	1.20	1.46	1.57	1.92	1.48	0.48	0.34	0.49	
NO	0.32	0.56	0.31	0.47	0.39	1.80	0.62	2.16	
HCN	0.90	0.79	0.89	0.94	1.53	0.78	0.57	1.01	
Benzene	1.16	1.06	1.14	1.19	1.12	0.87	0.74	0.73	
Toluene	1.06	0.93	1.07	1.18	1.20	1.02	0.79	0.84	
Isoprene	1.22	0.84	1.03	1.68	1.86	0.58	0.73	0.62	
Phenol	1.34	0.72	1.01	1.37	1.63	0.69	0.60	0.57	
m/p-Cresol	1.12	0.77	0.91	1.32	1.43	0.83	0.69	0.81	
o-Cresol	1.20	0.83	0.96	1.27	1.42	0.77	0.70	0.77	
Formaldehyde	1.56	1.58	2.14	1.12	1.36	0.18	0.26	0.24	
Acetaldehyde	1.05	0.97	1.16	1.14	1.11	0.86	1.07	0.97	
Acetone	1.07	1.01	0.99	0.90	0.97	0.99	0.91	1.08	
Butanone (MEK)	1.13	1.09	1.04	0.97	1.10	0.96	0.89	0.92	
Crotonaldehyde	1.17	1.03	1.15	1.11	1.36	0.78	0.86	0.84	
NNK	0.33	0.30	0.16	0.31	1.63	0.93	1.41	1.67	

NNN	0.09	0.07	0.02	0.10	0.23	0.69	0.26	1.93	
NAT	0.16	0.15	0.06	0.26	0.45	0.74	0.21	2.04	
NAB	0.19	0.17	0.17	0.34	0.52	0.71	0.30	2.05	
4-Aminobiphenyl	0.73	0.70	0.50	0.96	1.09	1.68	0.93	0.82	
3-Aminobiphenyl	0.74	0.74	0.53	1.04	1.25	1.40	0.91	0.90	
2-Aminonaphthalene	1.00	0.87	0.63	1.37	1.62	1.02	0.84	0.64	
1-Aminonaphthalene	0.95	1.00	0.71	1.44	1.57	0.97	0.95	0.57	
Resorcinol	1.61	0.61	1.28	2.04	1.49	0.49	0.61	0.32	
Hydroquinone	1.46	0.55	1.34	1.29	1.33	0.64	1.24	0.34	
Catechol	1.57	0.81	1.37	1.59	1.55	0.44	0.55	0.31	
Acrolein	1.22	1.12	1.33	1.19	1.32	0.62	0.72	0.78	
NFDPM	1.14	0.75	0.94	1.26	1.41	0.83	0.75	0.80	
Nicotine	1.38	0.73	0.97	1.77	1.46	0.52	0.37	0.58	
CO	1.04	0.82	0.90	1.04	1.06	1.01	0.98	1.00	
ALL Hoffmann's (29)	**0.99**	**0.79**	**0.90**	**1.10**	**1.24**	**0.84**	**0.72**	**0.93**	

Appendix 3:

Correlations (r) between levels of Hoffmann analytes and toxicity as measured by various biological assays (calculated from puff specific data, EC 50 [puffs/L] vs. analyte/per puff).
Numbers in bold indicate statistical significance.
Brackets indicate positive (+) or negative (-) correlation between analyte and toxicity.

Analyte per puff	Ames mutagenicity rev/puff	Vapour phase EC 50 (NRU)	Whole smoke EC 50 (NRU)	Vapour phase EC 50 (MTS)
BaP	**-0.726 (-)**	**-0.780 (+)**	**-0.877 (+)**	**-0.830 (+)**
Nitric oxide	**0.755 (+)**	**0.761 (-)**	**0.902 (-)**	**0.852 (-)**
HCN	0.466	0.426	**0.712 (-)**	0.509
Benzene	0.309	0.156	0.269	0.279
Toluene	**0.823 (+)**	**0.619 (-)**	0.517	**0.664 (-)**
Isoprene	0.297	-0.058	-0.429	-0.183
Phenol	0.224	0.155	-0.203	0.021
m/p-cresol	**0.909 (+)**	**0.660 (-)**	0.429	**0.608 (-)**
o-cresol	**0.781 (+)**	0.549	0.308	0.485
Formaldehyde	**-0.965 (-)**	**-0.846 (+)**	**-0.734 (+)**	**-0.857 (+)**
Acetaldehyde	0.383	0.000	0.068	0.038
Acetone	**0.633 (+)**	0.472	0.595	0.533
2-Butanone	0.423	0.220	0.302	0.269
Crotonaldehyde	0.034	-0.333	-0.387	-0.388
total TSNA	**0.677 (+)**	**0.731 (-)**	**0.824 (-)**	**0.779 (-)**
total Aromatic Amines	**0.860 (+)**	**0.672 (-)**	0.327	**0.646 (-)**
Resorcinol	-0.439	-0.528	**-0.733 (+)**	**-0.628 (+)**
Hydroquinone	-0.095	-0.221	-0.426	-0.301
Catechol	**-0.709 (-)**	**-0.666 (+)**	**-0.778 (+)**	**-0.758 (+)**

Acrolein	**-0.657 (-)**	**-0.910 (+)**	**-0.730 (+)**	**-0.907 (+)**